Myofibrils are thinner and electron microscopy shows a disrupted M-line and shifted H-zones. This phenotype was rescued by precise P-element excision using a transgene fly stock carrying a transposase. Non-flight muscles are not affected by the mutation. Obscurin RNAi lines driven with an IFM specific Gal4 driver lead to a flightless phenotype, and the specific reduction of obscurin IFM isoforms. Electron microscopy shows the phenotype is more severe than in the P-element mutant. Co-immunoprecipitation showed that obscurin is associated with myosin. It is likely that obscurin is needed for normal alignment and symmetry of thick filaments. In yeast two-hybrid screens, a 400 kDa protein, MASK, was identified as a binding partner of obscurin kinase 2. MASK co-localises with obscurin in the M-line. MASK RNAi lines show a flightless phenotype. A possible binding partner for obscurin kinase 1 is ball, a kinase of unknown specificity. MASK and ball can both be linked to signalling pathways involved in muscle development.

Workshop 1: Advanced Single Molecule Fluorescence Techniques in Vitro and in Vivo

1040-Wkshp
Single-Molecule Analysis of Transcription
Richard Ebright[1], Shimon Weiss[2], Anirban Chakraborty[1], Dongye Wang[1], You Korlann[2], Achillefs Kapanidis[2], Emmanuel Margeat[2].
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We are using single-molecule fluorescence resonance energy transfer to define fundamental aspects of transcription initiation, elongation, and termination.
In published work, we have shown that initial transcription proceeds through a "scrunching'' mechanism, in which RNA polymerase (RNAP) remains fixed on promoter DNA and pulls downstream DNA past its active center. We have shown further that putative alternative mechanisms for RNAP-active-center translocation in initial transcription, involving "transient excursions" of RNAP or "inchworming" of RNAP, do not occur. The results support a model in which a stressed intermediate, with DNA-unwinding stress and DNA-compaction stress, is formed during initial transcription, and in which accumulated stress is used to drive breakage of RNAP-promoter interactions during promoter escape.
In unpublished work, we are assessing opening and closing of the RNAP active-center-cleft, movements of modules of sigma relative to RNAP in transcription initiation, movements of modules of the RNAP active center in transcription elongation, and movements of RNAP relative to DNA in transcription termination.
In further unpublished work, carried out in support of these studies, we have developed reagents and procedures that permit incorporation of a fluorescent probe at any position of interest within a transcription complex.

1041-Wkshp
In vitro and in vivo; kinesin and myosin moving one (or a few) at a time
Paul Selvin.
University of Ilinois, Urbana, Urbana, IL, USA.

1042-Wkshp
In-Vivo Super-Resolution Microscopy by Structured Illumination
Mats G.L. Gustafsson.
HHMI Janelia Farm Research Campus, Ashburn, VA, USA.
Periodically structured illumination light can extend the resolution of fluorescence microscopy beyond the classical limit through spatial frequency mixing. The amount of resolution extension, set by the spatial frequency of the illumination pattern, is normally about a factor of two, because the pattern frequency is limited by the diffraction in the same way as the conventional resolution.
Dramatically greater resolution extension is possible, however, if a nonlinearity can be introduced between the incoming illumination intensity and the outgoing emission rate, because such a nonlinearity can create harmonics of the illumination frequency. Reversible photo-switching of fluorophores constitutes one promising form of such nonlinearity.
Structured-illumination microscopy typically uses data reconstruction algorithms that assume that the entire data set represents a single unchanging structure. It has therefore been largely confined to fixed, unmoving samples. If a data set can be acquired in a time that is short compared to sample movement speeds, however, live imaging becomes possible. Here we present live imaging with ~100 nm lateral resolution at multi-Hz rates for hundreds of time frames, using linear structured illumination with a rapid pattern-generating system in the TIRF mode.

1043-Wkshp
Advanced Fluorescence Microscopy Of Single, Living Cells: Using Optical Proteomics To Study Native Biochemistry One Molecule At A Time
Mark C. Leake.
Oxford University, Oxford, United Kingdom.
What is the molecular basis of the cell? How do single-molecule properties in a living organism scale up to effect whole-organism functionality? Can we bridge our gap in understanding between molecular biology and cell science in a rational, predictive context? These questions pose some of the hardest and most fundamental challenges to the future of biological research. Full understanding of processes in living organisms is only achievable if all molecular interactions are considered, though to date the shear complexity of biological systems has caused precise single-molecule experimentation to be far too demanding, instead focusing on studies of single systems using relatively crude bulk ensemble-average measurements. What I will discuss are some experiments that are leading us to being able to monitor several biological systems simultaneously in a single living, functioning cell using ultra-sensitive single-molecule techniques.

1044-Wkshp
Elucidating Mechanisms in Complex Systems by Multi-wavelength Single-molecule Fluorescence
Jeff Gelles.
Brandeis Univ, Waltham, MA, USA.
Many biological systems function through multiple non-covalent interactions between different proteins or nucleic acids. Even when only a few different kinds of macromolecules are involved, it is often true that a large number of different non-covalent complexes can form. This combinatorial complexity can make using conventional biochemical approaches to elucidate the kinetic mechanisms of these systems intractably difficult. Multi-wavelength single-molecule fluorescence is powerful approach to mechanistic analysis of these complex systems. By following individual molecules, this method can define reaction pathways and measure kinetics even in mixtures as complex as whole cell extracts. This talk will illustrate this approach with examples taken from basic processes in molecular biology including transcription and pre-mRNA splicing.

Workshop 2: Channelopathies of Nerve and Muscle

1045-Wkshp
Mechanistic Diversity for Channelopathies of Brain and Skeletal Muscle
Stephen C. Cannon.
UT Southwestern Medical Center, Dallas, TX, USA.
Mutations in the coding sequence of voltage-gated ionic channels are known to cause a wide variety of diseases affecting muscle and brain. Biophysical studies on the functional consequences of these defects are now revealing an equally diverse spectrum of mechanisms that underlie the disruption of cellular excitability, synaptic transmission, or neuronal survival. This Workshop on Channelopathies highlights recent advances in understanding the mechanistic connection between altered channel behavior and disease pathogenesis. New knock-in mouse models of Familial Hemiplegic Migraine illustrate how subtle gain-of-function changes in P/Q-type CaV2.1 channels enhance excitatory synaptic transmission and promote cortical spreading depression. A transmembrane protein linked to Familial Alzheimer Disease (presenilin) has recently been shown to form an unconventional Ca2+ leak channel that accounts for 80% of the divalent conductance of the ER. Finally, new insights have emerged in the past two years on a possible common pathomechanism by which mutations in either NaV1.4 or CaV1.1 channels of skeletal muscle may cause periodic paralysis. In both cases, mutations are clustered at arginine residues of the S4 voltage-sensor domain. Mutant channels conduct small ‘‘omega’’ currents through a voltage-regulated gating pore and may be the source of the inward current that renders affected fibers susceptible to sustained depolarized shifts during attacks of weakness.

1046-Wkshp
Neuronal calcium channels and migraine
Daniela Pietrobon.
Dept of Biomedical Sciences and CNR Inst. of Neuroscience, University of Padova, Padova, Italy.
Migraine is a common disabling brain disorder of unknown etiology. A subtype of migraine with aura (familial hemiplegic migraine type 1: FHM1) is caused